

VI. *The Prothoracic Gland of Dicranura vinula, and other Notes.* By OSWALD H. LATTER, Assistant Master at Charterhouse, formerly Tutor of Keble College.

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PLATE V.

i. THE FUNCTION, AND STRUCTURE, AND HOMOLOGIES OF PROTHORACIC GLANDS.

THE present communication is the outcome and continuation of my previous investigations, of which accounts are to be found in *Trans. Ent. Soc. Lond.*, 1892, pp. 287-292, and 1895, pp. 399-412. Being desirous of tracing the source of the potassium hydroxide solution by the aid of which the imago softens the cocoon at the time of emergence, and having already shown that this solution is stored in a special dorsal diverticulum at the anterior extremity of the mid-gut of the pupa, I proceeded in the summer of 1895 to inquire into the nature of the alkaline fluid which is abundantly present in the alimentary canal of the larva. Twenty larvæ in their last stage were procured, their alimentary canals were dissected out, and thrown into deci-normal hydrochloric acid of known weight and there minced, while the remainder of their bodies was likewise placed in another vessel containing hydrochloric acid of similar strength.

I will deal with these latter first, for they led me to more complete results.

On the day following the dissections above mentioned I was astonished to find at the bottom of the beaker containing the hydrochloric acid into which the bodies of the larvæ had been thrown, a thick gelatinous deposit covering all the bottom to a depth of about $\frac{1}{4}$ inch. This deposit was semi-opaque, pale yellow in colour and sufficiently firm to allow of its being removed *en masse* from the beaker and of being lifted by one side without breaking or indeed bending

excessively. Examination of this deposit revealed shreds of the walls of the silk glands scattered throughout the sheet. It at once occurred to me that if this effect was produced by the action of hydrochloric acid upon the contents of the silk glands, it was possible that the formic acid secreted by the prothoracic ("neck") glands, as described by Klemensiewicz (8) and Poulton (12, 13) might perhaps in a similar way give rise to the peculiar horny and non-fibrous character of the silk of the cocoon of this species. This suspicion was rendered the more strong by the fact that the sheet taken from the hydrochloric acid when exposed to the air and allowed to dry assumed in the course of a few hours an appearance and consistency very similar indeed to that of a *Vinula* cocoon spun without fragments of wood, etc. Unfortunately I was unable, in consequence of want of material, to proceed further with the subject in 1895, and accordingly was obliged to defer the work till the summer of 1896, when I procured a large number of larvæ from Messrs. Edmonds of Windsor. My first step was to determine whether formic acid behaved towards the excised silk glands in the same manner as hydrochloric acid. Accordingly the silk glands of a fully grown larva were dissected out and at once placed in formic acid, and there cut up into several pieces. In the course of a few hours the whole mass had swollen greatly and had set into a tolerably firm and nearly transparent jelly. On exposing this jelly to the air so as to permit evaporation, shrinkage took place, and eventually there was left a hard horny mass very closely resembling a cocoon both in texture and appearance. I may here add that I treated the silk glands of several other species, notably of *L. cossus* and of the larva of the Japan Silk-Moth, *A. pernyi*, with formic acid in the same way but with totally different results. The glands of *L. cossus* remained flaccid, but became whiter and more opaque, while those of *A. pernyi* became very tough and much harder. It is thus evident that a special relation exists between the composition of the undischarged silk of *D. vinula* and formic acid, and it is further probable that the chemical composition of the silk of various species is very far from constant.

I next endeavoured to ascertain whether the larva did as a matter of fact employ its formic acid when con-

structing its cocoon. Prof. Poulton (12) has already pointed out that the freshly-made cocoon while still moist is strongly acid, which observation I can amply confirm. Moreover, if a larva is watched when spinning it is at once seen that the aperture of the prothoracic gland is brought into close proximity with that of the silk glands, and further that the former is constantly moist. I interrupted a larva in this occupation, and by means of a fine roughened pair of forceps caught the end of the thread projecting from the silk-gland aperture; I then held the larva against a glass slide which was flooded with formic acid, and drew the thread into the acid; the larva continued to discharge the silk as I gently pulled the thread out. The first portion of the thread, which was dry and hard when I seized it, remained unaltered, but the portions following passed at once into the formic acid, swelled a little, and adhered firmly to the glass and also to those portions of the thread with which they came in contact, for I was able to draw out several inches of silk and to coil it up and down the slide. I then removed the glass slide, and substituted another free from formic acid. The silk which was drawn out on to this slide adhered feebly to the glass, did not swell, nor did the various lengths adhere to one another, but I was able to lift from the slide a fine thread of fibrous silk in no way partaking of the gelatinous character which had been assumed by that which had been led into formic acid.

In order to put the matter beyond doubt I tried to prevent the formic acid from being employed in the construction of the cocoon. By means of blotting-paper and fine glass tubes I endeavoured to remove all the formic acid from the glands of a few larvæ which had just begun to spin up. The handling necessary for this so disturbed the larvæ that in most cases they died without pupating, only one of them succeeded in pupating, but not one of them made any further attempts at spinning. I therefore abandoned this method in favour of neutralising the acid. Two larvæ ready to spin were placed in a glass vessel whose sides and bottom had been moistened with a solution of sodium carbonate; no materials were given for incorporation with the cocoon. Both these made cocoons which were very similar to those usually constructed in the absence of wood-chips, etc., but at those

places where the cocoons touched the glass numerous fine silky threads could be seen which had not become agglutinated to the main mass of the cocoon. Six other larvæ of like age were placed in other glass vessels and provided with fragments of blotting-paper which had previously been soaked in solution of sodium carbonate and then dried. Five of these made cocoons incorporating the paper fragments and pupated. Each of these cocoons presents a very large number of loose silk fibres on the external surface, and especially in the immediate neighbourhood of the alkaline scraps of paper, but on the inner surfaces and where the external surface touches the glass the texture is smooth and horny as usual.

These experiments conclusively prove that the formic acid secreted by the larva is utilised not only for defensive purposes during larval life, but also for giving a special character to the silk of the cocoon, whereby the latter is rendered extremely tenacious to any foreign substance brought in contact with it and also extraordinarily hard, waterproof, and completely closed against all aggressors. When the exposed position of the cocoon on a tree trunk is considered it will at once be seen that this remarkable relation between the silk of this species and formic acid constitutes a wonderfully complete adaptation to the specific needs.

It would be interesting to endeavour to decide which of these two functions of the prothoracic gland is phylogenetically the older. Personally, I am inclined to think that the defensive is the more ancient; for the gland is as fully developed and relatively of the same size in the freshly-hatched larva as in that ready to pupate. This evidence is certainly not conclusive; and, indeed, the question can hardly be settled until more is known of the function of the prothoracic glands of other larvæ—a point to which I hope to direct my attention this year.

Passing now to the structure of the prothoracic glands in this species. By way of preliminary I wish to enter an emphatic protest against the use of such terms as "neck" or "chin" glands in reference to these structures. The word "neck" assuredly refers to the region between the head and thorax—a region which I have not yet seen in any lepidopterous larva; and to many minds an idea of dorsal position is associated with the word, whereas those glands are ventral, while "chin," though

preferable to the former term, conveys an entirely misleading idea as to the true position in the prothoracic segment.

Klemensiewicz (8) has already given a very full description and figure of the gland and its appendages, while in this country Poulton (12, 13) has described and figured the external appearances. The existence of this gland has long been known, but the literature dealing with it and with analogous structures appears to be so little known in this country that I have thought it advisable to give a short history of the work that has been done, and to append a bibliographic list to this paper.

De Geer (3). It is very singular that the work of this author has been so very generally overlooked. I have found no mention of him in any of the authorities cited, yet so long as one hundred and fifty years ago—1745 and 1746—the main features of the organ now under discussion were accurately described. De Geer does not name his species, but describes it as “*la Chenille à double queue du Saule.*” He points out and figures the exact position of the aperture of the gland, the existence of “*le reservoir intérieur,*” the power of ejecting the liquid to a distance, the freedom of the sac from all but tracheal attachments and that of its duct, and the presence of two pairs of eversible lateral tubes covered all over with “*un grand nombre de poils courts.*” These lateral tubes, however, he fancies to be perforated by numerous minute holes, appearing similar to the “*rose*” of a watering-can, and suggests, though confesses he has not seen the process, that the fluid is ejected through these foramina. He also mentions the irritating nature of the fluid, having received part of a discharge “*dans mon œil droit, où cette liqueur me causait d’abord une douleur cuisante et comme ardente mais qui, à ma grande satisfaction, ne duroit pas longtemps.*” The defensive value of the apparatus is mentioned, and its “*odeur très forte et pénétrante, entièrement semblable à celle de la liqueur que jettent les grands Fourmis des bois.*”

Very full details are given as to the habits of the larva in using this gland, and it is clear that this early author made a very complete study of the subject. I am indebted to Professor Poulton for directing my attention to de Geer’s work, and to my friend Mr. W. Hatchett

Jackson for very kindly furnishing me with an abstract of these memoirs.

Rengger (16), writing in 1817, incorrectly asserts that the gland discharges its secretion by means of two eversible muscular tubes, from which the secretion is ejected.

Von Siebold (20), in 1848, mentions that the larva can defend itself by discharges of a corrosive liquid.

I can find no further contribution of any importance before that of Klemensiewicz (8), 1882. This author gives a very full and, in the main, accurate account and figures of the structures, and suggests that the secretion is formic rather than acetic acid; at the same time, he rightly assigns the actual discharge to the action of the muscles of the body-wall.

Poulton (12), 1886, proved the acid to be formic acid, but erroneously described the eversible processes. In 1887 (13) this error was corrected by him, but, as pointed out by Schäffer (18) and (19), he only described the external appearances, and thus fell into the error of regarding the median sac as a storage organ; whereas the true sac is not eversible, and is the actual secretory gland. The same author in 1887 (14) and (15) determined the strength of the formic acid solution, and proved its protective value by experiments on natural foes.

Since the specimens I have examined, both by dissection and series of microscopic sections, exhibit several differences to those described by the above authors, I give my own results in full. The horizontal slit-like aperture which is visible upon the red margin of the prothorax leads into a shallow cavity, which I shall refer to as the "vestibule." This is compressed antero-posteriorly, but of slightly greater width than the slit laterally. From nearly the right and left corners of the vestibule there pass backward and outward (in the retracted condition) a pair of hollow tubes capable of eversion. Each tube, after a very short course, divides into two main parts, about 1.3 mm. long when extracted and preserved in alcohol, which end blindly (*vide* Fig. 1, t^1 , t^2); from the point of bifurcation a very short third tube is given off (t^3). Of the two tubes the anterior, when everted (t^1), is slightly the longer, and its cavity is divided into two for a very short distance at the apex (*vide* Fig. 6): to the apex of this tube is attached a pair of retractor muscles (*r.m.*¹),

while but a single retractor ($r m^2$) is attached to the apex of t^2 , a second, however, being attached to this tube at a point about half-way along its length ($r m^{2'}$). These tubes are lined with chitin of the ordinary kind covering the external surface of the body, but towards their extremities are provided with a number of fine needle-like bristles, arising in tufts from the summits of low knobs (*vide* Figs. 1 and 3). Their epithelia have none of the usual characters of glandular cells, and I am confident that they are not of this nature. I can form no positive opinion of the function of these eversible tubes.

Schäffer (19) suggests that these side pouches are reservoirs for the acid, and that the bristles serve to keep the tubes from being crushed by the body-fluids pressing upon them. It seems to me unlikely that such relatively small structures should be the reservoirs for so large a gland, whose own internal capacity appears ample for the purpose; nor am I convinced that these tubes do as a matter of fact ever contain acid. It seems highly improbable that they are of any service in the discharge of the formic acid, for they are not everted by the larva unless external pressure is put upon its body, and even then not until the acid has been already discharged. This at least is my experience, and Professor Poulton in conversation confirms me, though Klemensiewicz (8) asserts that they are everted just before the discharge. It is possible that they may be of service in dire necessity when the larva is seized by a foe in somewhat increasing the terrifying aspect of the victim, and affording a somewhat larger surface for the evaporation of the formic acid in close proximity to the aggressor. There are no protractor muscles to these tubes, their eversion being solely due to pressure of liquid.

The vestibule itself is provided with powerful protractor and retractor muscles. The former are situated along the anterior side of the vestibule on either side of the median region from which they are absent; they are inserted about half-way up the side of the vestibule and take origin from the internal surface of the body wall anterior to the slit aperture; on each side, right and left, these muscles form an almost continuous sheet, which can, however, be separated into some five or six main bundles which are visible to the naked eye (*vide* Figs. 1 and 4, $p m v$). These muscles come into action prior to the

discharge of acid and cause evagination of the vestibule, which then appears as a green band externally; at the same time they bring the orifice of the true gland (*vide infra*) up to the external surface. The retractor muscles are arranged in four groups of three each; a pair are inserted a little to the right and left respectively of the middle line on to the extreme posterior wall of the vestibule (Fig. 1, *rmv*). In the resting position of the larva these muscle bands have a curved course to their origin from the ventral body-wall, but in the attitude assumed under alarm the course would be almost, if not entirely straight. The other retractors of the vestibule are inserted on to the extreme lateral corners of the vestibule (Fig. 1, *lrmv*) and originate from the ventrolateral body-wall.

From the middle of the posterior wall of the vestibule there leads backward a short cylindrical or funnel-shaped duct (Fig. 1, *d*), which issues from the large flask-shaped glandular sac (Fig. 1, *gs*). The duct is strengthened and doubtless kept open by a number of stout chitinous hoops, which project some distance in the lumen of the duct (*vide* Fig.).

The gland sac in a full-grown larva may be 8 mm. long, 5 mm. wide, and about 3 mm. deep. Except for tracheal attachments it lies almost free in the body cavity; its anterior end by which it is connected to the duct lies just behind the first thoracic ganglion and (generally) to the right of the nerve cord which is thrust over slightly to the left in consequence (*vide* Fig. 5); the bulk of the gland is dorsal to the nerve cord between it and the alimentary canal.

The minute structure of this gland has been described by Klemensiewicz (8), and I have not much to add to his description. The epithelial cells that secrete the chitin lining the vestibule gradually pass into those of the gland without any sharp line of demarcation, all stages of transition being found along the course of the discharging duct. The cells of this duct get larger in passing towards the gland, and there is a corresponding increase of nucleus. The cells are arranged in more or less circular (spiral?) rows round the duct, and each row produces a well marked ridge of chitin projecting into the lumen of the duct; the anterior ridges are very small, and they gradually become more pronounced in passing backwards

(vide Figs. 7, 8, 9). At the posterior end of the duct, and in the gland proper, the cells are very large with fluted sides, and the secreted chitin is extremely thin and often difficult to identify, though I believe it to be always present, the plasma of the cells is relatively far more abundant, and presents a curiously striated appearance at its free border owing to vacuolation of this portion of the cell, at the same time the nuclei are of great size and branch out into numerous blunt processes corresponding with the external flutings. The general appearance both of the cell plasma and of the nucleus is very similar to that of the cells of the silk-glands.

I now pass on to consider the homologies of this sternal prothoracic gland and of similar structures.

Bernard (1) has endeavoured to show that the tracheæ arose from setiparous sacs of a Chætopod ancestor, and considers that the stink-glands of *Julus* among the Myriapoda represent the glands of parapodial setæ, while coxal glands may represent acicular gland sacs. Packard (10) has expressly stated his opinion that these sternal glands of lepidopterous larvæ are the homologues of coxal glands of other Arthropods, and, if I understand him aright, that the same view is tenable of the lateral and dorsal glands of many species of larvæ. In the following discussion I shall confine my attention to the sternal glands, and chiefly to those of Lepidoptera. It is very striking to note how large a number of the segments of the entire body possess, in various species, structures which may with reason be regarded as serial homologues of the gland seen in *D. vinula*. A tabular arrangement will show this most clearly.

Head segment. 1. Oil (stink) glands opening at base of mandibles of *C. ligniperda*.*

3. Silk glands.†

Thorax. 1. All species of *Dicranura* (*Cerura*), *Marcurocampa marthesia*, Packard (11), *Schizura concinna* (id.); species of *Lacosoma*, *Astyanax*, *Danima*, *Nola*, *Hyperchiria*, *Hyponomeuta*, and *Plusia*, Schäffer (19).

* Salivary glands; glandules of Filippi. I prefer not to assign these to any definite segments.

† Bütschli, Zeit. wiss. Zool. Bd. 20, 1870.

- Thorax.* 2. *Perophora melsheimeri*, Schäffer (19),
Lacosoma, 5 species of Nolidæ.
 Many Hemiptera.
3. *P. melsheimeri*, *Lacosoma*, 5 species of
 Nolidæ. Imago of Bed-bug.
- Abdomen.* 1. *Hyperchiria varia*, Dimmock (4). *H. io*,
 Packard (11). *Hemileuca maia* (id.).
6. *H. maia*.
7. *H. varia*; *H. io*.
8. *H. maia*.

In *Campodea staphylinus* similar glands have been described by Packard (10) in abdominal segments 1 to 8, and in 1 to 7 in *Madulis*.*

It is thus seen that in all the segments of the body, with the exception of the difficult segments of the head, glands of this nature are present within the limits of the Insecta. A closer examination of the accounts given by various authors affords very striking support to Bernard's view alluded to above.

Packard (11) describes the larva of *Perophora melsheimeri* as possessing small sternal tubercles on the 2nd and 3rd thoracic segments. There are four of these structures on each segment, two on each side of the middle line. The square area enclosed by these four has in its centre the mouth of an eversible gland. The same author describes similar structures on all the thoracic segments of *Lacosoma*, and mentions further the interesting fact that, according to C. D. Ash, the posterior pair on the 3rd segment are "armed at the end with several crowded spine-like granulations." The correspondence of the above structures with the prothoracic sternal gland of *D. vinula* is very obvious, especially when the lateral tubes of the latter are in the everted condition.

Other species present a most striking series of transitions towards the actual conditions found in *D. vinula*. On the 6th and 8th abdominal segments of *Hemileuca maia*, according to Packard (10), are "indications of aborted glands (which are well developed in segments 1 and 7 of the abdomen) encroached upon by two or three pale flattened minute spiniferous warts, which are wanting (invaginated? O. H. L.) in segments 1 and 7."

The larva of *Astyanax archippus* possesses in the 1st

* *Vide* Carrière, Arch. f. mikr. Anatomie, xxxv., 1890.

thoracic segment an "eversible soft tubercle covered with short hairs." That of *Nola strigula* has glandular sternal apertures in the 2nd and 3rd thoracic segments, and carries "tubiform appendages near the thoracic feet," Packard (10).

Schäffer (19) describes the sternal prothoracic gland of *Hyponomeuta evonymella* as being divisible into anterior and posterior portions, provided with two retractor muscles, and furnished with a spiny internal lining.

The dorsal abdominal glands of *Porthesia similis* are stated by Klemensiewicz (8) to secrete a clear, odourless fluid, and the interior of each cell to form a separate projection into the lumen of the gland.

Professor Poulton has told me in conversation, and given me permission to mention the fact that the secretion of the sternal prothoracic gland of *Stauropus fagi* is acid.

A large number of other larvæ and imagines have been described by various authors, particularly Schäffer (18), Haase (6), Packard (10, 11), as possessing similar glandular structures, but the details of their descriptions are not sufficient for purposes of comparison. The above statements, however, make it clear that there exists a fairly complete series which commences with projecting setiparous warts or tubercles surrounding a glandular opening, and leads up to invaginated spiny (setiparous) tubes placed laterally to the opening of the median gland. This evidence appears to me sufficient to justify the supposition that these lateral structures are directly derived from setiparous projections of a Chætopod ancestor, and I would maintain that the spines now present in the lateral tubes of *D. vinula* and other species are the actual representatives of original setæ. Bernard (1) suggests that the ventral parapodium is represented by the tracheate leg, that the acicular gland sac of the dorsal parapodium gave rise to the trachea itself of the Hexapod, that the scattered tracheæ of *Peripatus* are derived from ordinary setiparous glands, and that the stink-glands of *Julus* found origin in the glands of parapodial setæ, the coxal glands, perhaps, in acicular gland sacs. The relations which I have described above, especially when taken in connection with the results of other observers lead me to the conclusion that the glands under discussion are the homologues of the coxal glands and of the acicular gland sacs of Chætopods, while the lateral appendages (spiny pro-

jections or tubes) are groups of parapodial setæ. It is noticeable as pointing to similarity of origin of these glands and tracheal tubes that in many cases the cells are spoken of as forming distinct chitinous projections into the cavity of the gland and its duct. This is notably the case in the duct of the gland of *D. vinula*, and is very suggestive of the well-known spiral thickenings of tracheal tubes; at the same time attention may be called to the branching nuclei of the cells of these glands and of those of the silk-glands which have been claimed as homologous with tracheæ (Bütschli, *loc. cit.*), though much importance cannot be attached to this feature.

Should the significance here claimed for these structures prove to be correct, they will go far towards establishing the primitive nature of the eruciform larva, of which many observers are already in favour.

ii. THE PRESENCE OF POTASSIUM HYDROXIDE IN THE ALIMENTARY CANAL.

In my communications already referred to I have shown that the imago of *D. vinula* discharges potassium hydroxide from a sac in connection with the alimentary canal. The digestive fluid of the larva being strongly alkaline rendered it probable that this fluid was of the same nature. In order to test this I examined the digestive system and the rest of the body with a view to finding potassium salts. Twenty larvæ were employed for the purpose; their alimentary canals were placed in dilute hydrochloric acid, and the remainder of their bodies in another lot of the same acid. In both cases I was able to determine by the platinum perchloride method the presence of potassium, which was also confirmed by spectroscopic examination. In the alimentary canal I could find no sure indication of any other salt than the hydroxide, but the remainder of the body appeared to contain traces of the carbonate as well. An unfortunate accident prevented me from making a reliable quantitative analysis.

iii. A CORRECTION.

I stated in *Trans. Ent. Soc. Lond.*, 1895, p. 403, that near the hinder end of the mesenteron of the freshly-emerged imago a short wide tube opened from the gut

cavity into the body cavity. I find that this is an abnormal occurrence, and though present in all the imagines that I examined in 1894-5, is due to an unnatural obstacle (a glass tube) having been substituted for the natural cocoon. I believe that the unusual efforts made by the imago in endeavouring to escape from such an artificial confinement causes rupture of the thin wall of a bladder connected with the hind end of the gut. In those imagines which have emerged from true cocoons there is such a bladder charged with excretions of a brownish-red colour, but in a few that I caused to emerge in glass tubes I found the wide tube that I previously described, and have no doubt that the explanation I now give is the correct one.

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